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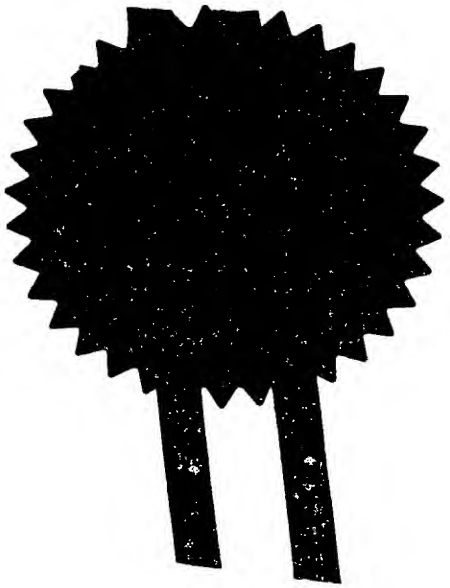

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Signed A W Brewer.

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1. Your reference — SMW/LP5631395

2. Patent application number *(The Patent Office will fill in this part)* — **9717192.0**

13 AUG 1997

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

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Patents ADP number *(if you know it)* 05714201

If the applicant is a corporate body, give the country/state of its incorporation — UNITED KINGDOM

4. Title of the invention — GENETIC CONTROL OF PLANT GROWTH AND DEVELOPMENT

5. Name of your agent *(if you have one)* — MEWBURN ELLIS

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

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Patents ADP number *(if you know it)* — 109006

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GENETIC CONTROL OF PLANT GROWTH AND DEVELOPMENT

This invention relates to the genetic control of growth and/or development of plants and the cloning and expression of genes involved therein. More particularly, the invention relates to the cloning and expression of the *Rht* gene of *Triticum Aestivum*, and homologues from other species, and use of the genes in plants.

An understanding of the genetic mechanisms which influence growth and development of plants, including flowering, provides a means for altering the characteristics of a target plant. Species for which manipulation of growth and/or development characteristics may be advantageous includes all crops, with important examples being the cereals, rice and maize, probably the most agronomically important in warmer climatic zones, and wheat, barley, oats and rye in more temperate climates. Important crops for seed products are oil seed rape and canola, maize, sunflower, soyabean and sorghum. Many crops which are harvested for their roots are, of course, grown annually from seed and the production of seed of any kind is very dependent upon the ability of the plant to flower, to be pollinated and to set seed. In horticulture, control of the timing of growth and development, including flowering, is important. Horticultural plants whose flowering may be controlled include lettuce, endive and vegetable brassicas including cabbage, broccoli and cauliflower, and carnations and geraniums. Dwarf plants on the one hand and over-size, taller plants on the other may be advantageous and/or desirable in various horticultural and agricultural

contexts, further including trees, plantation crops and grasses.

Recent decades have seen huge increases in wheat grain yields due to the incorporation of semi-dwarfing *Rht* homeoalleles into breeding programmes. These increases have enabled wheat productivity to keep pace with the demands of the rising world population. Previously, we described the cloning of the *Arabidopsis gai* alleles (unpublished International patent application PCT/GB97/00390 filed 12 February 1997, John Innes Centre Innovations Limited, the full contents of which are incorporated herein by reference) which, like *Rht* mutant alleles, confers a semi-dominant dwarf phenotype and a reduction in responsiveness to the plant growth hormone gibberellin (GA). *gai* encodes a mutant protein (gai) which lacks a 17 amino acid residue segment found near the N-terminus of the wild-type (GAI) protein. The sequence of this segment is highly conserved in a rice cDNA sequence (EST). Here we show that this cDNA maps to a short section of the overlapping cereal genome maps known to contain the *Rht* loci, and that we have used the cDNA to isolate the *Rht* genes of wheat. That genomes as widely diverged as those of *Arabidopsis* and *Triticum* should carry a conserved sequence which, when mutated, affects GA responsiveness, indicates a role for that sequence in GA signalling that is conserved throughout the plant kingdom. Furthermore, cloning of *Rht* permits its transfer to many different crop species, with the aim of yield enhancement as great as that obtained previously with wheat.

The introduction of semi-dwarfing *Rht* homeoalleles (originally known as Norin 10 genes, derived from a Japanese variety, Norin 10) into elite bread-wheat breeding lines was one of the most significant contributors to the so-called "green revolution" (Gale *et al*, 1985. Dwarfing genes in wheat. In: Progress in Plant Breeding, G.E. Russell (ed) Butterworths, London pp 1-35). Wheat containing these homeoalleles consistently out-yield wheats lacking them, and now comprise around 80% of the world's wheat crop. The biological basis of this yield-enhancement appears to be two-fold. Firstly, the semi-dwarf phenotype conferred by the *Rht* alleles causes an increased resistance to lodging (flattening of plants by wind/rain with consequent loss of yield). Secondly, these alleles cause a reallocation of photoassimilate, with more being directed towards the grain, and less towards the stem (Gale *et al*, 1985). These properties have major effects on wheat yields, as demonstrated by the fact that UK wheat yields increased by over 20% during the years that *Rht*-containing lines were taken up by farmers.

The *rht* mutants are dwarfed because they contain a genetically dominant, mutant *rht* allele which compromises their responses to gibberellin (GA, an endogenous plant growth regulator) (Gale *et al*, 1976. Heredity 37; 283-289). Thus the coleoptiles of *rht* mutants, unlike those of wild-type wheat plants, do not respond to GA applications. In addition, *rht* mutants accumulate biologically active GAs to higher levels than found in wild-type controls (Lenton *et al*, 1987. Gibberellin insensitivity and depletion in wheat -

consequences for development. In: Hormone action in Plant Development - a critical appraisal. GV Haod, JR Lenton, MB Jackson and RK Atkin (eds) Butterworths, London pp 145-160). These properties (genetic dominance, reduced GA-responses, and high endogenous GA levels) are common to the phenotypes conferred by mutations in other species (*D8/D9* in maize; *gai* in *Arabidopsis*), indicating that these mutant alleles define orthologous genes in these different species, supported further by the observation that *D8/D9* and *Rht* are syntenous loci in the genomes of maize and wheat.

According to a first aspect of the present invention there is provided a nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide with Rht function. The term "Rht function" indicates ability to influence the phenotype of a plant like the *Rht* gene of *Triticum*. "*Rht* function" may be observed phenotypically in a plant as inhibition, suppression, repression or reduction of plant growth which inhibition, suppression, repression or reduction is antagonised by GA. *Rht* expression tends to confer a dwarf phenotype on a plant which is antagonised by GA. Overexpression in a plant from a nucleotide sequence encoding a polypeptide with *Rht* function may be used to confer a dwarf phenotype on a plant which is correctable by treatment with GA.

Also according to an aspect of the present invention there is provided a nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide with ability to

confer a *rht* mutant phenotype upon expression. *rht* mutant plants are dwarfed compared with wild-type, the dwarfing being GA-insensitive.

Herein, "Rht" (capitalised) is used to refer to the wild-type function, while "rht" (uncapitalised) is used to refer to mutant function.

Many plant growth and developmental processes are regulated by specific members of a family of tetracyclic diterpenoid growth factors known as gibberellins (GA) (Hooley, *Plant Mol. Biol.* 26, 1529-1555 (1994)). By gibberellin or GA is meant a diterpenoid molecule with the basic carbon-ring structure shown in Figure 6 and possessing biological activity, i.e. we refer to biologically active gibberellins.

Biological activity may be defined by one or more of stimulation of cell elongation, leaf senescence or elicitation of the cereal aleurone α-amylase response. There are many standard assays available in the art, a positive result in any one or more of which signals a test gibberellin as biologically active (Hoad et al., *Phytochemistry* 20, 703-713 (1981); Serebryakov et al., *Phytochemistry* 23, 1847-1854 (1984); Smith et al., *Phytochemistry* 33, 17-20 (1993)).

Assays available in the art include the lettuce hypocotyl assay, cucumber hypocotyl assay, and oat first leaf assay, all of which determine biological activity on the basis of ability of an applied gibberellin to cause elongation of the respective tissue. Preferred assays are those in which the test composition is applied to a gibberellin-deficient plant. Such preferred assays include treatment of dwarf GA-deficient

Arabidopsis to determine growth, the dwarf pea assay, in which internode elongation is determined, the Tan-ginbozu dwarf rice assay, in which elongation of leaf sheath is determined, and the *d5*-maize assay, also in which elongation of leaf sheath is determined. The elongation bioassays measure the effects of general cell elongation in the respective organs and are not restricted to particular cell types.

Further available assays include the dock (*Rumex*) leaf senescence assay and the cereal aleurone α-amylase assay. Aleurone cells which surround the endosperm in grain secrete α-amylase on germination, which digests starch to produce sugars then used by the growing plant. The enzyme production is controlled by GA. Isolated aleurone cells given biologically active GA secrete α-amylase whose activity can then be assayed, for example by measurement of degradation of starch.

Structural features important for high biological activity (exhibited by GA_1, GA_3, GA_4 and GA_7) are a carboxyl group on C-6 of B-ring; C-19, C-10 lactone; and β-hydroxylation at C-3. β-hydroxylation at C-2 causes inactivity (exhibited by GA_8, GA_{29}, GA_{34} and GA_{51}). *rht* mutants do not respond to GA treatment, e.g. treatment with GA_1, GA_3 or GA_4.

Treatment with GA is preferably by spraying with aqueous solution, for example spraying with 10^{-4}M GA_3 or GA_4 in aqueous solution, perhaps weekly or more frequently, and may be by placing droplets on plants rather than spraying. GA may be applied dissolved in an organic solvent such as ethanol or

acetone, because it is more soluble in these than in water, but this is not preferred because these solvents have a tendency to damage plants. If an organic solvent is to be used, suitable formulations include 24ηl of 0.6, 4.0 or 300mM GA_3 or GA_4 dissolved in 80% ethanol. Plants, e.g. *Arabidopsis*, may be grown on a medium containing GA, such as tissue culture medium (GM) solidified with agar and containing supplementary GA.

Nucleic acid according to the present invention may have the sequence of a wild-type *Rht* gene of *Triticum* or be a mutant, derivative, variant or allele of the sequence provided. Preferred mutants, derivatives, variants and alleles are those which encode a protein which retains a functional characteristic of the protein encoded by the wild-type gene, especially the ability for plant growth inhibition, which inhibition is antagonised by GA, or ability to confer on a plant one or more other characteristics responsive to GA treatment of the plant. Other preferred mutants, derivatives, variants and alleles encode a protein which confers a *rht* mutant phenotype, that is to say reduced plant growth which reduction is insensitive to GA, i.e. not overcome by GA treatment. Changes to a sequence, to produce a mutant, variant or derivative, may be by one or more of addition, insertion, deletion or substitution of one or more nucleotides in the nucleic acid, leading to the addition, insertion, deletion or substitution of one or more amino acids in the encoded polypeptide. Of course, changes to the nucleic acid which make no difference to the encoded amino acid sequence

are included.

A preferred nucleotide sequence for a *Rht* gene is one which encodes the RHT amino acid sequence shown in Figure 3b, especially a *Rht* coding sequence shown in Figure 3a. A preferred *rht* mutant lacks part or all of the 17 amino acid sequence underlined in Figure 3b, and/or part or the sequence DVAQKLEQLE, which immediately follows the 17 amino acid sequence underlined in Figure 3b.

The present invention also provides a nucleic acid construct or vector which comprises nucleic acid with any one of the provided sequences, preferably a construct or vector from which polypeptide encoded by the nucleic acid sequence can be expressed. The construct or vector is preferably suitable for transformation into a plant cell. The invention further encompasses a host cell transformed with such a construct or vector, especially a plant cell. Thus, a host cell, such as a plant cell, comprising nucleic acid according to the present invention is provided. Within the cell, the nucleic acid may be incorporated within the chromosome. There may be more than one heterologous nucleotide sequence per haploid genome. This, for example, enables increased expression of the gene product compared with endogenous levels, as discussed below.

A construct or vector comprising nucleic acid according to the present invention need not include a promoter or other regulatory sequence, particularly if the vector is to be used to introduce the nucleic acid into cells for recombination into the genome. However, in one aspect the present invention

provides a nucleic acid construct comprising a *Rht* or *rht* coding sequence (which includes homologues from other than *Triticum*) joined to a regulatory sequence for control of expression, the regulatory sequence being other than that naturally fused to the coding sequence and preferably of or derived from another gene.

Nucleic acid molecules and vectors according to the present invention may be as an isolate, provided isolated from their natural environment, in substantially pure or homogeneous form, or free or substantially free of nucleic acid or genes of the species of interest or origin other than the sequence encoding a polypeptide able to influence growth and/or development, which may include flowering, eg in *Triticum Aestivum* nucleic acid other than the *Rht* coding sequence. The term "nucleic acid isolate" encompasses wholly or partially synthetic nucleic acid.

Nucleic acid may of course be double- or single-stranded, cDNA or genomic DNA, RNA, wholly or partially synthetic, as appropriate. Of course, where nucleic acid according to the invention includes RNA, reference to the sequence shown should be construed as reference to the RNA equivalent, with U substituted for T.

The present invention also encompasses the expression product of any of the nucleic acid sequences disclosed and methods of making the expression product by expression from encoding nucleic acid therefor under suitable conditions in suitable host cells. Those skilled in the art are well able to construct vectors and design protocols for expression and

recovery of products of recombinant gene expression. Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. For further details see, for example, *Molecular Cloning: a Laboratory Manual*: 2nd edition, Sambrook et al, 1989, Cold Spring Harbor Laboratory Press. Transformation procedures depend on the host used, but are well known. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in *Protocols in Molecular Biology*, Second Edition, Ausubel et al. eds., John Wiley & Sons, 1992. Specific procedures and vectors previously used with wide success upon plants are described by Bevan, Nucl. Acids Res. (1984) 12, 8711-8721), and Guerineau and Mullineaux, (1993) Plant transformation and expression vectors. In: Plant Molecular Biology Labfax (Croy RRD ed) Oxford, BIOS Scientific Publishers, pp 121-148. The disclosures of Sambrook et al. and Ausubel et al. and all other documents mentioned herein are incorporated herein by reference.

Expression as a fusion with a polyhistidine tag allows purification of Rht or rht to be achieved using Ni-NTA resin available from QIAGEN Inc. (USA) and DIAGEN GmbH (Germany). See Janknecht et al., *Proc. Natl. Acad. Sci. USA* 88, 8972-8976 (1991) and EP-A-0253303 and EP-A-0282042. Ni-NTA resin has

high affinity for proteins with consecutive histidines close to the N- or C- terminus of the protein and so may be used to purifiy histidine-tagged Rht or rht proteins from plants, plant parts or extracts or from recombinant organisms such as yeast or bacteria, e.g. *E. coli*, expressing the protein.

Purified Rht protein, e.g. produced recombinantly by expression from encoding nucleic acid therefor, may be used to raise antibodies employing techniques which are standard in the art. Antibodies and polypeptides comprising antigen-binding fragments of antibodies may be used in identifying homologues from other species as discussed further below.

Methods of producing antibodies include immunising a mammal (eg human, mouse, rat, rabbit, horse, goat, sheep or monkey) with the protein or a fragment thereof. Antibodies may be obtained from immunised animals using any of a variety of techniques known in the art, and might be screened, preferably using binding of antibody to antigen of interest. For instance, Western blotting techniques or immunoprecipitation may be used (Armitage et al, 1992, Nature 357: 80-82). Antibodies may be polyclonal or monoclonal.

As an alternative or supplement to immunising a mammal, antibodies with appropriate binding specificty may be obtained from a recombinantly produced library of expressed immunoglobulin variable domains, eg using lambda bacteriophage or filamentous bacteriophage which display functional immunoglobulin binding domains on their surfaces; for instance see WO92/01047.

Antibodies raised to a Rht, or rht, polypeptide can be

used in the identification and/or isolation of homologous polypeptides, and then the encoding genes. Thus, the present invention provides a method of identifying or isolating a polypeptide with Rht function or ability to confer a *rht* mutant phenotype, comprising screening candidate polypeptides with a polypeptide comprising the antigen-binding domain of an antibody (for example whole antibody or a fragment thereof) which is able to bind an *Triticum Aestivum* Rht or rht polypeptide, or preferably has binding specificity for such a polypeptide, such as having the amino acid sequence shown in Figure 4.

Candidate polypeptides for screening may for instance be the products of an expression library created using nucleic acid derived from an plant of interest, or may be the product of a purification process from a natural source.

A polypeptide found to bind the antibody may be isolated and then may be subject to amino acid sequencing. Any suitable technique may be used to sequence the polypeptide either wholly or partially (for instance a fragment of the polypeptide may be sequenced). Amino acid sequence information may be used in obtaining nucleic acid encoding the polypeptide, for instance by designing one or more oligonucleotides (e.g. a degenerate pool of oligonucleotides) for use as probes or primers in hybridisation to candidate nucleic acid, as discussed further below.

A further aspect of the present invention provides a method of identifying and cloning *Rht* homologues from plant species other than *Triticum* which method employs a nucleotide

sequence derived from any shown in Figure 2 or Figure 3a. Sequences derived from these may themselves be used in identifying and in cloning other sequences. The nucleotide sequence information provided herein, or any part thereof, may be used in a data-base search to find homologous sequences, expression products of which can be tested for *Rht* function. Alternatively, nucleic acid libraries may be screened using techniques well known to those skilled in the art and homologous sequences thereby identified then tested.

For instance, the present invention also provides a method of identifying and/or isolating a *Rht* or *rht* homologue gene, comprising probing candidate (or "target") nucleic acid with nucleic acid which encodes a polypeptide with *Rht* function or a fragment or mutant, derivative or allele thereof. The candidate nucleic acid (which may be, for instance, cDNA or genomic DNA) may be derived from any cell or organism which may contain or is suspected of containing nucleic acid encoding such a homologue.

In a preferred embodiment of this aspect of the present invention, the nucleic acid used for probing of candidate nucleic acid encodes an amino acid sequence shown in Figure 3b, a sequence complementary to a coding sequence, or a fragment of any of these, most preferably comprising a nucleotide sequence shown in Figure 3a.

Alternatively, as discussed, a probe may be designed using amino acid sequence information obtained by sequencing a polypeptide identified as being able to be bound by an antigen-binding domain of an antibody which is able to bind a

Rht or rht polypeptide such as one with the Rht amino acid sequence shown in Figure 3b.

Preferred conditions for probing are those which are stringent enough for there to be a simple pattern with a small number of hybridizations identified as positive which can be investigated further. It is well known in the art to increase stringency of hybridisation gradually until only a few positive clones remain.

As an alternative to probing, though still employing nucleic acid hybridisation, oligonucleotides designed to amplify DNA sequences from *Rht* genes may be used in PCR or other methods involving amplification of nucleic acid, using routine procedures. See for instance "PCR protocols; A Guide to Methods and Applications", Eds. Innis et al, 1990, Academic Press, New York.

Preferred amino acid sequences suitable for use in the design of probes or PCR primers are sequences conserved (completely, substantially or partly) between *Rht* genes.

On the basis of amino acid sequence information, oligonucleotide probes or primers may be designed, taking into account the degeneracy of the genetic code, and, where appropriate, codon usage of the organism from the candidate nucleic acid is derived. In particular, primers and probes may be designed using information on conversed sequences apparent from, for example, Figure 3 and/or Figure 4.

Where a full-length encoding nucleic acid molecule has not been obtained, a smaller molecule representing part of the full molecule, may be used to obtain full-length clones.

Inserts may be prepared for example from partial cDNA clones and used to screen cDNA libraries. The full-length clones isolated may be subcloned into vectors such as expression vectors or vectors suitable for transformation into plants. Overlapping clones may be used to provide a full-length sequence.

The present invention also extends to nucleic acid encoding *Rht* or a homologue obtainable using a nucleotide sequence derived from Figure 2 or Figure 3a, and such nucleic acid obtainable using one or more, e.g. a pair, of primers including a sequence shown in Figure 5.

Also included within the scope of the present invention are nucleic acid molecules which encode amino acid sequences which are homologues of the polypeptide encoded by *Rht* of *Triticum*. A homologue may be from a species other than *Triticum*.

Homology may be at the nucleotide sequence and/or amino acid sequence level. Preferably, the nucleic acid and/or amino acid sequence shares homology with the sequence encoded by the nucleotide sequence of Figure 3a, preferably at least about 50%, or 60%, or 70%, or 80% homology, most preferably at least 90%, 92%, 95% or 97% homology. Nucleic acid encoding such a polypeptide may preferably share with the *Triticum Rht* gene the ability to confer a particular phenotype on expression in a plant, preferably a phenotype which is GA responsive (i.e. there is a change in a characteristic of the plant on treatment with GA), such as the ability to inhibit plant growth where the inhibition is antagonised by GA. As

noted, *Rht* expression in a plant may affect one or more other characteristics of the plant. A preferred characteristic that may be shared with the *Triticum Rht* gene is the ability to complement a *Rht* null mutant phenotype in a plant such as *Triticum*, such phenotype being resistance to the dwarfing effect of paclobutrazol. The *slender* mutant of barley maps to a location in the barley genome equivalent to that of *Rht* in the wheat genome. Such mutant plants are strongly paclobutrazol resistant. The present inventors believe that the *slender* barley mutant is a null mutant allele of the orthologous gene to wheat *Rht*, allowing for complementation of the barley mutant with the wheat gene. Ability to complement a *slender* mutant in barley may be a characteristic of embodiments of the present invention.

Some preferred embodiments of polypeptides according to the present invention (encoded by nucleic acid embodiments according to the present invention) include the 17 amino acid sequence which is underlined in Figure 3b, or a contiguous sequence of amino acids residues with at least about 10 residues with similarity or identity with the respective corresponding residue (in terms of position) in 17 amino acids which are underlined in Figure 3b, more preferably 11, 12, 13, 14, 15, 16 or 17 such residues, and/or the sequence DVAQKLEQLE, or a contiguous sequence of amino acids with at least about 5 residues with similarity or identity with the respective corresponding residue (in terms of position) within DVAQKLEQLE, more preferably 6, 7, 8 or 9 such residues. Further embodiments include the 27 amino acid sequence

DELLAALGYKVRASDMADVAQKLEQLE, or a contiguous sequence of amino acids residues with at least about 15 residues with similarity or identity with the respective corresponding residue (in terms of position) within this sequence, more preferably 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or 26 such residues.

As is well-understood, homology at the amino acid level is generally in terms of amino acid similarity or identity. Similarity allows for "conservative variation", i.e. substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine. Similarity may be as defined and determined by the TBLASTN program, of Altschul *et al.* (1990) *J. Mol. Biol.* 215: 403-10, which is in standard use in the art. Homology may be over the full-length of the *Rht* sequence of Figure 3b, or may more preferably be over a contiguous sequence of 10 amino acids compared with DVAQKLEQLE, and/or a contiguous sequence of 17 amino acids, compared with the 17 amino acids underlined in Figure 3b, and/or a contiguous sequence of 27 amino acids compared with DELLAALGYKVRASDMADVAQKLEQLE,or a longer sequence, e.g. about 30, 40, 50 or more amino acids, compared with the amino acid sequence of Figure 3b and preferably including the underlined 17 amino acids and/or DVAQKLEQLE.

At the nucleic acid level, homology may be over the full-length or more preferably by comparison with the 30 nucleotide coding sequence within the sequence of Figure 3a and encoding the sequence DVAQKLEQLE and/or the 51 nucleotide coding

polypeptide which includes an amino acid sequence which is a mutant, allele, derivative or variant sequence of the *Rht* amino acid sequence of the species *Triticum Aestivum* shown in Figure 3b, or is a homologue of another species or a mutant, allele, derivative or variant thereof, wherein said mutant, allele, derivative, variant or homologue differs from the amino acid sequence shown in Figure 3b by way of insertion, deletion, addition and/or substitution of one or more amino acids, as obtainable by producing transgenic plants by transforming plants which have a *Rht* null mutant phenotype, which phenotype is resistance to the dwarfing effect of paclobutrazol, with test nucleic acid, causing or allowing expression from test nucleic acid within the transgenic plants, screening the transgenic plants for those exhibiting complementation of the *Rht* null mutant phenotype to identify test nucleic acid able to complement the *Rht* null mutant, deleting from nucleic acid so identified as being able to complement the *Rht* null mutant a nucleotide sequence encoding the 17 amino acid sequence underlined in Figure 3b or a contiguous 17 amino acid sequence in which at least 10 residues have similarity or identity with the respective amino acid in the corresponding position in the 17 amino acid sequence underlined in Figure 3b, more preferably 11, 12, 13, 14, 15, 16 or 17, and/or a nucleotide sequence encoding DVAQKLEQLE or a contiguous sequence of 10 amino aicds with at least about 5 (more preferably 6, 7, 8 or 9) which have similarity or identity with the corresponding residue in the sequence DVAQKLEQLE.

A cell containing nucleic acid of the present invention represents a further aspect of the invention, particularly a plant cell, or a bacterial cell.

The cell may comprise the nucleic acid encoding the enzyme by virtue of introduction into the cell or an ancestor thereof of the nucleic acid, e.g. by transformation using any suitable technique available to those skilled in the art.

Also according to the invention there is provided a plant cell having incorporated into its genome nucleic acid as disclosed.

The present invention also provides a plant comprising such a plant cell.

Also according to the invention there is provided a plant cell having incorporated into its genome a sequence of nucleotides as provided by the present invention, under operative control of a regulatory sequence for control of expression. A further aspect of the present invention provides a method of making such a plant cell involving introduction of a vector comprising the sequence of nucleotides into a plant cell and causing or allowing recombination between the vector and the plant cell genome to introduce the sequence of nucleotides into the genome.

A plant according to the present invention may be one which does not breed true in one or more properties. Plant varieties may be excluded, particularly registrable plant varieties according to Plant Breeders' Rights. It is noted that a plant need not be considered a "plant variety" simply because it contains stably within its genome a transgene,

expression, and therefore growth and/or development of the plant according to preference. Furthermore, mutants and derivatives of the wild-type gene may be used in place of the endogenous gene. The inserted gene may be foreign or exogenous to the host cell, e.g. of another plant species.

The principal characteristic which may be altered using the present invention is growth.

According to the model of the *Rht* gene as a growth repressor, under-expression of the gene may be used to promote growth, at least in plants which have only one endogenous gene conferring *Rht* function (not for example *Arabidopsis* which has endogenous homologues which would compensate). This may involve use of anti-sense or sense regulation. Taller plants may be made by knocking out *Rht* or the relevant homologous gene in the plant of interest. Plants may be made which are resistant to compounds which inhibit GA biosynthesis, such as paclobutrazol, for instance to allow use of a GA biosynthesis inhibitor to keep weeds dwarf but let crop plants grow tall.

Over-expression of a *Rht* gene may lead to a dwarf plant which is correctable by treatment with GA, as predicted by the *Rht* repression model.

Since *rht* mutant genes are dominant on phenotype, they may be used to make GA-insensitive dwarf plants. This may be applied for example to any transformable crop-plant, tree or fruit-tree species. It may provide higher yield/reduced lodging like Rht wheat. In rice this may provide GA-insensitive rice resistant to the Bakane disease, which is a problem in Japan and elsewhere. Dwarf ornamentals may be of

value for the horticulture and cut-flower markets. Sequence manipulation may provide for varying degrees of severity of dwarfing, GA-insensitive phenotype, allowing tailoring of the degree of severity to the needs of each crop-plant or the wishes of the manipulator. Over-expression of *rht*-mutant sequences is potentially the most useful.

A second characteristic that may be altered is plant development, for instance flowering. In some plants, and in certain environmental conditions, a GA signal is required for floral induction. For example, GA-deficient mutant *Arabidopsis* plants grown under short day conditions will do not flower unless treated with GA: these plants do flower normally when grown under long day conditions. *Arabidopsis gai* mutant plants show delayed flowering under short day conditions: severe mutants may not flower at all. Thus, for instance by *Rht* or *rht* gene expression or over-expression, plants may be produced which remain vegetative until given GA treatment to induce flowering. This may be useful in horticultural contexts or for spinach, lettuce and other crops where suppression of bolting is desirable.

The nucleic acid according to the invention may be placed under the control of an externally inducible gene promoter to place the *Rht* or *rht* coding sequence under the control of the user.

The term "inducible" as applied to a promoter is well understood by those skilled in the art. In essence, expression under the control of an inducible promoter is "switched on" or increased in response to an applied stimulus.

The nature of the stimulus varies between promoters. Some inducible promoters cause little or undetectable levels of expression (or no expression) in the absence of the appropriate stimulus. Other inducible promoters cause detectable constitutive expression in the absence of the stimulus. Whatever the level of expression is in the absence of the stimulus, expression from any inducible promoter is increased in the presence of the correct stimulus. The preferable situation is where the level of expression increases upon application of the relevant stimulus by an amount effective to alter a phenotypic characteristic. Thus an inducible (or "switchable") promoter may be used which causes a basic level of expression in the absence of the stimulus which level is too low to bring about a desired phenotype (and may in fact be zero). Upon application of the stimulus, expression is increased (or switched on) to a level which brings about the desired phenotype.

Suitable promoters include the Cauliflower Mosaic Virus 35S (CaMV 35S) gene promoter that is expressed at a high level in virtually all plant tissues (Benfey et al, 1990a and 1990b); the maize glutathione-S-transferase isoform II (GST-II-27) gene promoter which is activated in response to application of exogenous safener (WO93/01294, ICI Ltd); the cauliflower meri 5 promoter that is expressed in the vegetative apical meristem as well as several well localised positions in the plant body, eg inner phloem, flower primordia, branching points in root and shoot (Medford, 1992; Medford et al, 1991) and the *Arabidopsis thaliana LEAFY*

promoter that is expressed very early in flower development (Weigel et al, 1992).

The GST-II-27 gene promoter has been shown to be induced by certain chemical compounds which can be applied to growing plants. The promoter is functional in both monocotyledons and dicotyledons. It can therefore be used to control gene expression in a variety of genetically modified plants, including field crops such as canola, sunflower, tobacco, sugarbeet, cotton; cereals such as wheat, barley, rice, maize, sorghum; fruit such as tomatoes, mangoes, peaches, apples, pears, strawberries, bananas, and melons; and vegetables such as carrot, lettuce, cabbage and onion. The GST-II-27 promoter is also suitable for use in a variety of tissues, including roots, leaves, stems and reproductive tissues.

Accordingly, the present invention provides in a further aspect a gene construct comprising an inducible promoter operatively linked to a nucleotide sequence provided by the present invention, such as the *Rht* gene of *Triticum* a homologue from another plant species or any mutant, derivative or allele thereof. This enables control of expression of the gene. The invention also provides plants transformed with said gene construct and methods comprising introduction of such a construct into a plant cell and/or induction of expression of a construct within a plant cell, by application of a suitable stimulus, an effective exogenous inducer. The promoter may be the GST-II-27 gene promoter or any other inducible plant promoter.

When introducing a chosen gene construct into a cell,

following introduction of the nucleic acid into plant cells, optionally followed by regeneration into a plant, e.g. using one or more marker genes such as antibiotic resistance (see above).

Plants transformed with the DNA segment containing the sequence may be produced by standard techniques which are already known for the genetic manipulation of plants. DNA can be transformed into plant cells using any suitable technology, such as a disarmed Ti-plasmid vector carried by Agrobacterium exploiting its natural gene transfer ability (EP-A-270355, EP-A-0116718, NAR 12(22) 8711 - 87215 1984), particle or microprojectile bombardment (US 5100792, EP-A-444882, EP-A-434616) microinjection (WO 92/09696, WO 94/00583, EP 331083, EP 175966, Green *et al.* (1987) *Plant Tissue and Cell Culture*, Academic Press), electroporation (EP 290395, WO 8706614 Gelvin Debeyser - see attached) other forms of direct DNA uptake (DE 4005152, WO 9012096, US 4684611), liposome mediated DNA uptake (e.g. Freeman *et al. Plant Cell Physiol.* 29: 1353 (1984)), or the vortexing method (e.g. Kindle, *PNAS U.S.A.* 87: 1228 (1990d). Physical methods for the transformation of plant cells are reviewed in Oard, 1991, *Biotech. Adv.* 9: 1-11.

Agrobacterium transformation is widely used by those skilled in the art to transform dicotyledonous species. Recently, there has been substantial progress towards the routine production of stable, fertile transgenic plants in almost all economically relevant monocot plants (Toriyama, et al. (1988) *Bio/Technology* 6, 1072-1074; Zhang, *et al.* (1988) *Plant Cell Rep.* 7, 379-384; Zhang, *et al.* (1988) *Theor Appl*

Genet 76, 835-840; Shimamoto, *et al.* (1989) *Nature* 338, 274-276; Datta, *et al.* (1990) *Bio/Technology* 8, 736-740; Christou, *et al.* (1991) *Bio/Technology* 9, 957-962; Peng, *et al.* (1991) International Rice Research Institute, Manila, Philippines 563-574; Cao, *et al.* (1992) *Plant Cell Rep.* 11, 585-591; Li, *et al.* (1993) *Plant Cell Rep.* 12, 250-255; Rathore, *et al.* (1993) *Plant Molecular Biology* 21, 871-884; Fromm, *et al.* (1990) *Bio/Technology* 8, 833-839; Gordon-Kamm, *et al.* (1990) *Plant Cell* 2, 603-618; D'Halluin, *et al.* (1992) *Plant Cell* 4, 1495-1505; Walters, *et al.* (1992) *Plant Molecular Biology* 18, 189-200; Koziel, *et al.* (1993) *Biotechnology* 11, 194-200; Vasil, I. K. (1994) *Plant Molecular Biology* 25, 925-937; Weeks, *et al.* (1993) *Plant Physiology* 102, 1077-1084; Somers, *et al.* (1992) *Bio/Technology* 10, 1589-1594; WO92/14828). In particular, *Agrobacterium* mediated transformation is now emerging also as an highly efficient transformation method in monocots (Hiei *et al.* (1994) *The Plant Journal* 6, 271-282).

The generation of fertile transgenic plants has been achieved in the cereals rice, maize, wheat, oat, and barley (reviewed in Shimamoto, K. (1994) *Current Opinion in Biotechnology* 5, 158-162.; Vasil, *et al.* (1992) *Bio/Technology* 10, 667-674; Vain et al., 1995, *Biotechnology Advances* 13 (4): 653-671; Vasil, 1996, *Nature Biotechnology* 14 page 702).

Microprojectile bombardment, electroporation and direct DNA uptake are preferred where Agrobacterium is inefficient or ineffective. Alternatively, a combination of different techniques may be employed to enhance the efficiency of the transformation process, eg bombardment with Agrobacterium

coated microparticles (EP-A-486234) or microprojectile bombardment to induce wounding followed by co-cultivation with Agrobacterium (EP-A-486233).

Brassica napus transformation is described in Moloney *et al.* (1989) *Plant Cell Reports* 8: 238-242.

Following transformation, a plant may be regenerated, e.g. from single cells, callus tissue or leaf discs, as is standard in the art. Almost any plant can be entirely regenerated from cells, tissues and organs of the plant. Available techniques are reviewd in Vasil et al., *Cell Culture and Somatic Cel Genetics of Plants, Vol I, II and III, Laboratory Procedures and Their Applications*, Academic Press, 1984, and Weissbach and Weissbach, *Methods for Plant Molecular Biology*, Academic Press, 1989.

The particular choice of a transformation technology will be determined by its efficiency to transform certain plant species as well as the experience and preference of the person practising the invention with a particular methodology of choice. It will be apparent to the skilled person that the particular choice of a transformation system to introduce nucleic acid into plant cells is not essential to or a limitation of the invention, nor is the choice of technique for plant regeneration.

In the present invention, over-expression may be achieved by introduction of the nucleotide sequence in a sense orientation. Thus, the present invention provides a method of influencing a characteristic of a plant, the method comprising causing or allowing expression of nucleic acid according to

of protein from the target gene occurs. When the inserted gene is only part of the endogenous gene the number of under-expressing individuals in the transgenic population increases. The mechanism by which sense regulation occurs, particularly down-regulation, is not well-understood. However, this technique is also well-reported in scientific and patent literature and is used routinely for gene control. See, for example, See, for example, van der Krol *et al.*, (1990) *The Plant Cell* 2, 291-299; Napoli *et al.*, (1990) *The Plant Cell* 2, 279-289; Zhang *et al.*, (1992) *The Plant Cell* 4, 1575-1588, and US-A-5,231,020.

Thus, the present invention also provides a method of influencing a characteristic of a plant, the method comprising causing or allowing expression from nucleic acid according to the invention within cells of the plant. This may be used to influence growth.

Aspects and embodiments of the present invention will now be illustrated, by way of example, with reference to the accompanying figures. Further aspects and embodiments will be apparent to those skilled in the art. All documents mentioned in this text are incorporated herein by reference.

The following Figures are included herein:

Figure 1: Alignment of N-terminus predicted GAI amino acid sequence (Gai) with rice EST D39460 (0830), with a region of homology outlined in black.

Figure 2: DNA sequences from C15-1, 14a1 and 5a1.

Figure 2a shows a consensus DNA sequence cDNA C15-1 (obtained via single-pass sequencing).

Figure 2b shows data from original DNA sequencing runs from 14a1 (single-pass).

Figure 2c shows data from original DNA sequencing runs from 5a1 (single-pass).

Figure 3: Rht sequences.

Figure 3a shows a composite DNA sequence of wheat *Rht* gene derived from data in Figure 2, including coding sequence.

Figure 3b shows an alignment of the entire predicted Rht protein sequence encoded by the coding sequence of Figure 2 (rht) with the entire predicted GAI protein sequence of *Arabidopsis* (Gai). Regions of sequence identity are highlighted in black.

Figure 4: D39460 sequence.

Figure 4a shows DNA sequence (single-pass) of rice cDNA D39460. This cDNA is an incomplete, partial clone, missing the 3' end of the mRNA from which it is derived.

Figure 4b shows alignment of the entire predicted Rht protein sequence (wheat - encoded by the coding sequence of Figure 2) with that of GAI (Gai) and rice protein sequence predicted from DNA sequence in Figure 4a (Rice). Regions of amino acid identity are highlighted in black; some conservative substitutions are shaded.

Figure 5: Primers used for sequencing Rht clones (Figure 2).

Figure 6: The basic carbon-ring structure of gibberellins.

Previously, we cloned the *GAI* gene of *Arabidopsis* (PCT/GB97/00390). Comparison of the DNA sequences of the wild-type (*GAI*) and mutant (*gai*) alleles showed that *gai* encodes a mutant predicted protein product (gai) which lacks a segment of 17 amino acids from close to the N-terminus of the protein. Screening of the DNA sequence databases with the *GAI* sequence revealed the existence of a rice EST (D39460) which contains a region of sequence very closely related to that of the segment that is deleted from GAI in the gai protein. A comprison of the predicted amino acid sequences from the region DELLA to EQLE are identical in both sequences. The two differences (V/A; E/D) are conservative substitutions, in which one amino acid residue is replaced by another having very similar chemical properties. In addition, the region of identity extends beyond the boundary of the deletion region in the gai protein. The sequence DVAQKLEQLE is not affected by the deletion in gai, and yet is perfectly conserved between the GAI and D39460 sequences (Figure 1).

D39460 was used in low-stringency hybridization experiments to isolate hybridizing clones from wheat cDNA and genomic libraries. Several clones were isolated, including C15-1 and C15-10 (cDNAs), and 5a1 and 14a1 (genomic clones). Clone C15-1 has been used in gene mapping experiments. Nullisomic-tetrasomic analysis showed that clone C15-1 hybridizes to genomic DNA fragments derived from wheat chromosomes 4A, 4B and 4D. This is consistent with clone C15-1 containing *Rht* sequence, since the *Rht* loci map to the gorup 4 chromosomes. Furthermore, recombinant analysis using a

population segregating for the *Rht-D1b* (formerly *Rht2*) allele identified a hybridizing fragment that displayed perfect co-segregation with the mutant allele. This placed the genomic location of the gene encoding the mRNA sequence in cDNA C15-1 within a 2 cM segment (that was already known to contain *Rht*) of the group 4 chromosomes, and provides strong evidence that the cDNA and genomic clones do indeed contain the *Rht* gene.

Figure 2a gives the complete (single-pass) DNA sequence of cDNA C15-1. We have also obtained DNA sequence for C15-10; it is identical with that of C15-1, and is therefore not shown. Figures 2b and 2c show original data from individual sequencing runs from clones 14a1 and 5a1. The sequences shown in Figure 2 can be overlapped to make a composite DNA sequence, shown in Figure 3a. This sequence displays strong homology with that of *Arabidopsis GAI*, as revealed by a comparison of the amino acid sequence of a predicted translational product of the wheat sequence (Rht) with that of *GAI* (GAI), shown in Figure 3b. In particular, the predicted amino acid sequence of the presumptive *Rht* reveals a region of near-identity with GAI over the region that is missing in gai (Figure 4). Figure 4 reveals that the homology that extends beyond the gai deletion region in the rice EST is also conserved in Rht (DVAQKLEQLE), thus indicating that this region, in addition to that found in the gai deletion, is involved in GA signal-transduction. This region is not found in SCR, another protein that is related in sequence to GAI but which is not involved in GA signalling. The primers used in the above sequencing experiments are shown in Figure 5.

```
Gai .....MKRD  HHHHHQDKKT  MMMNEEDDGN  GMDELLAVLG  YKVRSSEMAD  VAQKLEQLEV  54
803 EAGGSSGGGS  SADMGSCKDK  VMAGAxGEEE  xVDELLAALG  YKVRSSDMAD  VAOKLEOLEM  60

Gai MMSNVQEDDL  SQLATETVHY  NPAELYTWLD
803 AMGMGGVTPP  AORMTGSCRT  WPRTKFI...
```

Figure 1

Consensus sequence of c-15-1

Cdna.Se Length: 1746 August 7, 1997 09:08 Type: N Check: 4797 ..

```
   1  CCCCGACGGT CGCGGCCGCG GCCAACGCGA CGCCCGCGCT GCCGGTCGTC
  51  GTGGTCGACA CGCAGGAGGC CGGGATTCGG CTGGTGCACG CGCTGCTGGC
 101  GTGCGCGGAG GCCGTGCAGC AGGAGAACCT CTCCGCCGCG GAGGCGCTGG
 151  TGAAGCAGAT ACCCTTGCTG GCCGCGTCCC AGGGCGGCGC GATGCGCAAG
 201  GTCGCCGCCT ACTTCGGCGA GGCCCTCGCC CGCCGCGTCT TCCGCTTCCG
 251  CCCGCAGCCG GACAGCTCCC TCCTCGACGC CGCCTTCGCC GACCTCCTCC
 301  ACGCGCACTT CTACGAGTCC TGCCCCTACC TCAAGTTCGC GCACTTCACC
 351  GCCAACCAGG CCATCCTGGA GGCGTTCGCC GGCTGCCGCC GCGTGCACGT
 401  CGTCGACTTC GGCATCAAGC AGGGGATGCA GTGGCCCGCA CTTCTCCAGG
 451  CCCTCGCCCT CCGTCCCGGC GGCCCTCCCT CGTTCCGCCT CACCGGCGTC
 501  GGCCCCCCGC AGCCGGACGA GACCGACGCC CTGCAGCAGG TGGGCTGGAA
 551  GCTCGCCCAG TTCGCGCACA CCATCCGCGT CGACTTCCAG TACCGCGGCC
 601  TCGTCGCCGC CACGCTCGCG GACCTGGAGC CGTTCATGCT GCAGCCGGAG
 651  GGCGAGGAGG ACCCGAACGA AGAXCCCGAX GTAATCGCCG TCAACTCAGT
 701  CTTCGAGATG CACCGGCTGC TCGCGCAGCC CGGCGCCCTG GAAAAGGTTC
 751  TTGGGCACCG TGCGCCCCCG TGCGGCCCAG AATTCXTCAC CGTGGTGGAA
 801  ACAGGAGGCA AATCACAACT CCGGCACATT CCTGGACCGC TTCACCGAGT
 851  CTCTGCACTA CTACTCCACC ATGTTCGATT CCCTCGAGGG CGGCAGCTCC
 901  GGCGGCGGCC CATCCGAAGT CTCATCGGGG GCTGCTGCTG CTCCTGCCGC
 951  CGCCGGCACG GACCAGGTCA TXTCCGAGGT GTACCTCGGC CGGCAGATCT
1001  GCAACGTGGT GGCCTGCGAG GGGGCGGAAC GCACAGAXCG CCACGAGACG
1051  CTGGGCCAGT GGCGGAACCG GCTGGGCAAC GCCGGGTTCG AGACCGTCCA
1101  CCTGGGCTCC AATGCCTACA AGCAGGCGAX CACGCTGCTG GCGCTCTTCG
1151  CCGGCGGCGA ACGGCTACAX GTGGAAGAAA AGGAAGGCTG CCTGACGCTG
1201  GGGTTGCACA CXCCCCCCTG ATTGCCACCT CGGCATGGCG CCTGGCCGGG
1251  CCGTGATCTC GCGAGTTTTG AACGCTGTAA GTACACATCG TGAGCATGGA
1301  GGACAACACA GCCCCGGCGG CCGCCCCGGC TCTCCGGCGA ACGCACGCAC
1351  GCACGCACTT GAAGAAGAAG AAGCTAAATG TCATGTCAGT GAGCGCTGAA
1401  TTGCAGCGAC CGGCTACGAT CGATCGGGCT ACGGGTGGTT CCGTCCGTCT
1451  GGCGTGAAGA GGTGGATGGA CGACGAACTC CGAGCCGACC ACCACCGGCA
1501  TGTAGTAATG TAATCCCTTC TTCGTTCCCA GTTCTCCACC GCCTCCATGA
```

```
1551  TCACCCGTAA AACTCCTAAG CCCTATTATT ACTACTATTA TGTTTAAATG

1601  TCTATTATTG CTATGTGTAA TTCCTCCAAC CGCTCATATC AAAATAAGCA

1651  CGGGCCGGAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA

1701  AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAA
```

Figure 2b (1)

```
  1  CGCGCAATGC TTAAGGTCXC CGCCTACTTC GGXGCAGGCC CTCGCCCGCC
 51  GCGTCTTCCG CTTCCGCCCG CAGCCGGACA GCTCCCTCCT CGACGCCGCC
101  TTCGCCGACC TCCTCCACGC GCACTTCTAC XAGTCCTGCC CCTACCTCAA
151  GTTCGCGCAC TTCACCGCCA ATTAGGCCAT CCTGGAGGCG TTCGCCGGCT
201  GCCGCCGCGT GCACGTCGTC GACTTCGGCA TCAAGCAGGG GATGCAGTGG
251  CCCGCACTTC TCCAGGCCCT CGCCCTCCGT CCCGGCGGCC CTCCCTCGTT
301  CCGCCTCACC GGCGTCGGCC CCCCGCAGCC GG
```

14 a 1 clone Sequenced with Rha-1 Primer

Figure 26-(2)

5

```
  1  ACCTCCTTCG TCGTCTXTXX GGTGGGGGCG CCAGGAGCTT ATGTGGTGGA
 51  GGXTGGCCCC XCCGGTCGCG ACCGCGXCCT ACGXGACGCC CGCGCTGCCG
101  GTCGTCGTGG TCGACACGCA GGAGGCCGGG ATTCGGXTGG TXCACGCGCT
151  GCTGGXGTGC GXGGAGXCCG TGCAGCAGGA GAACCTCTCC GCCGCGGAGG
201  CGCTXGTGAA GXAGATACCC XTGCTGGCCG AGTCCCAGGG CGGCGAGATG
251  XGCAAGGTXG CAGCTTACTT XGXAGAXGCC CTCGCCCGCX GAGTGATTCC
301  ACTTAXCGCC TGCAGCCGGA XAGCTCCGTC CTCGAAXCCG CXTTXGCCGA
351  CCTCCTCCAC GXGCACXTXT ACGAGTC
```

14a1 clone Sequenced with Rha-2 primer

```
  1  TAXTAGTCTC TCGGTGGGGG CGCCAGGAGC TCTXTGGTGG AGGCXGCCCC
 51  GCCGGTCGCG GCCGCGGCCA ACGCGACGCC CGCGCTGCCG GTCGTCGTGG
101  TCGACACGCA GGAGGCCGGG ATTCGGATGG TGCACGCGCT GXTGGCGTGC
151  GCGGAGGCCG TGAAACAGTT GAAGGXCCXC GCCTXXXXXC XCACAAXXTG
201  AAAGCCCCGX G
```

14 a 1 clone Sequenced with Rha-3 primer

Fig 2 b-(4)

```
  1  GGCTXCCXCC XCGTGCACGT CGTCGACTTC GGCATCAAGC ATGGGATGCA
 51  XTGGCXCGXA CTTCTCCAXG CCCTCGCCCT CCGTCCCGGC GGCCCTCCCT
101  CGTTCCGCCT CACCGGCGTC GGCCCCCCGC AGCCGGACGA GACCGACGCC
151  CTGCAXCAGG TGGGCTGGAA GCTCGCCCAG TTCGCGCACA CCATCCGCGT
201  CGACTTCCAX TACCGTGGCC TCGTCGCCGC CACGCTCGCG GACCTGGAGC
251  CGTTCATGCT GCAXCCGGAG GGCGAGGAGG ACCCGAACGA CGGAGCCCGA
301  GGTAATCGCC GTCAACTCAG TCTTCGAGAT GCACCGGGCT GCTCXCGCAX
351  CCCGGCGACX CTGGAAXAA
```

14 a 1 clone Sequenced with Rha-5 primer

Fig 2 b-(5)

```
  1  CAAGAXGCTA ATCACAACTC CGGCACATTC CTGGACCGCT TCACCGAGTC
 51  TCTGCAXTAC TACTCCACCA TGTTCGATTC CCTCGAGGGC GGCAGCTCCG
101  GCGGCGGCCC ATCCGAAGTC TCATCGGGGG CTGCTGCTGC TCCTGCCGCC
151  GCCGGCACGG ACCATGTCAT GTCCGAXGTG TACCTCGGCC GGCAGATCTG
201  CAACGTGGTG GCCTGCGAGG GGGCGGAGCG CACAXTAXCG CCACGCAGAC
251  XCTGGGCCAG TGGCGTGAAC CGGCTGGGCA ACGCCXGGTT CAXXXXCCGT
301  CCACCTGGGC TCCAATGCCT ACAATCAXGC XXXCACGCTG CTGGCGCCTC
351  TTCGCCC
```

14 a 1 clone Sequenced with Rha-7 primer

Figure 20-6

```
  1  TCGCCAXTCG GCATGGXGCC TGGCCGGGCC GTGATCTCGC GAGTTTTGAA
 51  CGCTGTAAGT ACACATCGTG AGCATGGAGG ACAACACAGC CCCGGCGGCC
101  GCCCCGGCTC TCCGGCGAAC GCACGCACGC ACGCACTTGG AAGAAGAAXA
151  AGCTAAATGT CATGTCAGTG AGCGCTGAAT TGCAACGACC GGCTACGATC
201  GATCGGGCTA CGGGTGGTTC CGTCCGTCTG GCGTGAAGAG GTGGATGGAC
251  GACGAACTCC GAXCCGACCA CCACCGGCAT GTAGTAATGT AATCCCTTCT
301  TCGTTCCCAG TTCTCCACCG CCTCCATGGA TCACCCGTAA AACTCCTAAG
351  CCCTAATTAT XXACTAACTA ATTATGTTTT AAAATGTTCT AATTAATTGG
401  CTATGTTGTA ATXCCTCCAA ACCGGCTCAT TTTCAAAXAT TAAGCCACGG
451  GCCCGGAACT TTGGTTTAAC AACCTCCCXA TTGXAAAATT XAAATXGAAA
501  TTTTTGGTTX C
```

14a1 clone Sequenced with Rha-8 primer

10

```
  1  GTTGGTGGXG GCGATTTGGG TACAAGGTGC GCGCCTCCGA CATGGXGGAX
 51  GTGGGGCAGA AGCTGGAGCA GXTCGAGATG GCCATGGGGA TGGGXGGCGT
101  GGGCGCTGGC GCCGCCCCTG ACGACAGGTT XGCCACCCGC XGGCCGCGGA
151  CACXGTGCAX TACAACCCCA CXGACXTGTC GTCTTGGGTC GAGAGCATGC
201  TGTCGGAGCT AAAXGAGCCG CXGCCGCCCC TCCCGCCCGC CCCGCAGCTC
251  AACGCCTCCA CCTCCTCCAC CGTCACGGGC AGCGGCGGCT ACTTCGATAA
301  CCCTCCCTG
```

14a1 clone Sequenced with Rht-9 primer

Figure 2b-⑧

```
  1  TGATGGXGGG AGXTTAXGGG TTAXAAATGT GGGGGAXTTC CGAAXXGGTG
 51  AGGAXATATX XTCAGAAGTT GGAGCAGATG AGAGATXGCT GATGGGGATA
101  GGGTAGGXGT GGGTGCCGGT GCXGCCCCCX AGGAXAGATT GGCCACCCAC
151  TTAGCAAGTG GAXACCGTGG ATTACXACCC CACAGACCTG TCGTGGTTGG
201  GTTTGAGAGC GTGGTGTGGG AGCTGAACGG GCXGCGGCGT GCCCCTCCCG
251  CCCGCCCCGC AGCTCAACGC CTCCACCTCC TCCACCGTAC ACGGGCAGCG
301  GCGGCTAGTT CGATCTCCCG CCCTCCGTCG ACTCCTCCAG CAGCATXTAX
351  GCGCTGCGGC CGATCCCCTX CCCAAGCXXG CGXGGXCCGA GCCGTGTAX
```

14a1 clone Sequenced with Rht-10 primer

Figure 26-(4)

12

```
  1  TTTCAXTTTC XTCCTTTTTT CTTCTTTTTC CAACCCCCGG CCCCCXGACC
 51  CTTGGATCCA AATCCCGAAC CCGCCCCCAG AACCXGGAAC CGAGGCCAAG
101  CAAAAGXTTT GCGCCAATTA TTGGCCAGAG ATAGATAGAG AGGCGAGGTA
151  GCTCGCGGAT CATGAAGCGG GAGTACCAGG ACGCCGGAGG GAGCGGCGGC
201  GGCGGTGGCG GCATGGGTTC GTCCGAGGAC AAGATGATGG TGTCGGCGGC
251  GGCGGGGGAG GGGGAGGAGG TGGACGAGCT GCTGGCGGCG CTCGGGTACA
301  AGGTGCGCGC CTCCGACATG GCGGACGTGG CGCAGAAGCT GGAGCAGCTC
351  GAGATGGCCA TGGGGATGGG CGGCGTGGGC GCCGGCGCCG CCCCCGACGA
401  CAGCTTCGCC ACCCACCTCG CCACGGACAC CGTGCAGTAC AACCXCCCXG
451  ACC
```

14a1 clone Sequenced with Rht-12 primer

Figure 2 b-(10)

13

```
  1  GGACGACGAC CTCCGAGCCG ACCACCACCG GCATGTAGTA ATGTAATCCC
 51  TTCTTCXTTC CCAGTXCTCC ACCGCCTCCA TGATCACCCG TAAAACTCCT
101  AAGCCCTATT ATTACTACTA TTATGTXTAA XTGTCTATTA TTGCTAXGTG
151  TAATTCCTCC AACCGCTCAT ATCAAAATAA GCACGGGCCG GACTTTGTTA
201  XCAGCTCCAA TGAGAATGAA ATGAATTTTG TACGCAAGGC ACGTCCAAAA
251  CTGGGCTGAG CTTTGTTCTG TTCTGTTATG TTCATGGTGC TCACTGCTCT
301  GATGAACATG ATGGTGCCTC CAATGGTGGC TTTGCAATTG TTGAAACGTT
351  TGGCTTGGGG GACTTGXGTG GGTGGGTGCA TGGGGATGAA TATTCACATC
401  XCCGGATTAA AATTAAGCCA TCCCGTTGGC CGTCCTTTGA ATAXCTTGCC
451  CXAAACGAAA TTTCCCCCXA TC
```

14 a 1 clone Sequenced with Rht-13 primer

Figure 26-(11)

14

```
  1  AAAXCCTAXA AXATATAGAG GCGATGTXGC XCCCCXATCA XXAACXGGAT
 51  TACXGXAACX CCXGAAGGAG CGGCGGCGGC GGTGGCAGCA TXGGCTCGTC
101  CGATGACAAA TATCATGGTG TCGGCGGCGG CGGGGGACGG GGAGGAGGTG
151  CACAACXTTT XGGCGGGACT CGXGTACCAC GTGXACGGTG CCGCXCTXGX
201  GGATXTGGCC CTXGAAGATG GGCCACCTCC AAA
```

14a1 clone Sequenced with Rht-14 primer

Figure 2b-(12)

15

```
  1  CGGCGGCCCC GTGGCGGCAT GGGCTCGTCC GAGGACXAGA TGATGGTGTC
 51  GGCGGCGGCG GGGGAXGGGG ATGATGTGGA CTATCTGCTG GCGGCGCTCG
101  GGTACAAGGT GCGCGCCTCC GACAGGCGGA GCCCGCGCAT AACTGGAGCC
151  GCTCGAGATG GCCXTGGGGA TXGGCGGCXT GGGCXCCXGC GCCTCCCCCG
```

14 a 1 clone Sequenced with Rht-15 primer

Figure 20-(13)


```
  1  TGGXGCTCGG GTGXCCCGTG CGCGCCTCCG ACATGGCGGG ACGTGGCGCA
 51  GAACTGGAGC AGCTCGAGAT GGCCATGGGG ATGGGCGGCG TGGGCGCCGG
101  CGCCGCCCCC GACGACAGCT TCGCCACCCA CCTCGCCACG GACACCGGCA
151  CACAACCCCA CCGACCTGTC GTCTTGGGTC GAGAGCATGC TGTCGGATCT
201  CXACGCGCCX CCGXCGCCCC TCCCGCCCGC
```

14a1 clone Sequenced with Rht-16 primer

Figure 2 (b)

```
    AXXTTGTXCX XXXTACATCC CATGXGCCGC GCXATGCTXA AGGTCGCCGC
 51 CTACTTCGGC GCAGGCCCTC GCCCGCCGCG TCTTCCGCTT CCGCCCGCAG
101 CCGGACAGCT CCCTCCTCGA CGCCGCCTTC GCCGACCTCC TCCACGCGCA
151 CTTCTACGAG TCCTGCCCCT ACCTCAAGTT CGCGCACTTC ACCGCCAACC
201 AGGCCATCCT GGAGGCGTTC GCCGGCTGCC GCCGCGTGCA CGTCGTCGAC
251 TTCGGCATCA AGCAGGGGAT GCAGTGGCCC GCACTTCTCC AGGCCCTCGC
301 CCTCCGTCCC GGCGGCCCTC CCTCGTTCCG CCTCACCGGC GTTCGGCCCC
351 CCGCAGCCGG ACGAXAACGA CGCCCTG
```

Sa1 clone Sequenced with Rha-1 primer

Figure 2C-(2)


```
  1  XTTCCCCGGC AGTTAAAAGC XTCCACTTCT TCCACCGTCA CGGGCAGCGG
 51  CGGXTACTTX GATCTCCCGC CCTCAGTCGA CTCCTCCAGC AGCATCTACG
101  CGCTGCGGCC GATCCCCTCC CCGGCCGGCG CGACGGCGCC GGCCGACCTG
151  TCCGCCGACT CCGTGCGGGA TCCCAAGCGG ATGCGCACTG GCGGGAGCAG
201  CACCTCGTCG TCATCCTCCT CATAXTCGTC TCTCGGTGGG GGCGCCAGGA
251  GCTCTGTGGT GGAGGCXGCC CCGCCGGTCG CGGCCGCGGC CAACGCGACG
301  CCCGCGCTGC CGGTCGTCGT GGTCGACACG CAGGAGGCCG GGATTCGGAT
351  GGTGCACGCG CTGXTGGCGT GCGCGGAGGC CGTGXAAGCA GTTXGAAGGG
401  CCTXCGCCGT GXATXXCGCA ACAAXXXGGA AGXCCX
```

5a1 clone Sequenced with Rha-3 primer

Figure 2C-(3)

```
  1  CAXCCCGCTG XTCGCCACCT CGGCATGGCG CCTGGCCGGG CCGTGATCTC
 51  GCGAGTTTTG AACGCTGTAA GTACACATCG TGAGCATGGA GGACAACACA
101  GCCCCGGCGG CGGCCCCGGC TCTCCGGCGA ACGCACGCAC GCACGCACTT
151  GAAGAAGAAG AAGCTAAATG TCATGTCAGT GAGCGCTGAA TTGCAXCGAC
201  CGGCTACGAT CGATCGGGCT ACGGGTGGTT CCGTCCGTCT GGCGTGAAGA
251  GGTGGATGGA CGACGAACTC CGAXCCGACC ACCACCGGCA TGTAGTAATG
301  TAATCCCTTC TTCGTTCCCA GTTTCTCCAC CGCCTCCATG ATCACCCCGT
351  AAAACTCCTA AGCCCTATXX XTTACTACXA TTAATGTTTT AAAXTGTTCT
401  AXTAATTGCT ATGXTGTTTA TTXCC
```

5a1 clone Sequenced with Rha-8 primer

Figure 2c - (4)

20

```
  1  TATCGAAGTA GCCGCCGCTG CCCXTGCACG GTGGAGGAGG TGGAGGCGTT
 51  GAGCTGCGGG GCGGGCGGGA GGGGCGGCGG CGGCACGTTX AGCTCCGACA
101  GCATGCTCTC GACCCAAAAC XACAGGTCGG TGGGGTTGTA GTGCACGGTG
151  TCCGTGGCGA GGGGGTGGCX AAXCTGTCGT CAGGGGCGGC GCCXGCGCCC
201  ACXCCGCCCA TCCCCATGGC CATCTCGAXC TGCTCCAGCT TCTGCGCCAC
251  TTCCXCCATG TCXGATGCGC GCXCCTTGTA CCCGA
```

Sa1 clone Sequenced with Rht-9 primer

Figure 2 C-(5)

21

```
  1  ACGGCGCGGX CCXCGCXXGC TTGGGAGGGG ATCGGCCGCA GCGCXTAXAT
 51  GCTGCTGGAG GAGTCGACGG AGGGCGGGAG ATCGAACTAG CCGCCGCTGC
101  CCGTGTACGG TGGAGGAGGT GGAGGCGTTG AGCTGCGGGG CGGGCGGGAG
151  GGGCAGCXGC TGCACGTTXA GCTCCCACAC CACGTCTCTC AACCCAACCA
201  CGACXCGTCT GTGGGGTXGT AATXCACGGT XTCCCTXGCT AXGTGGGTGG
251  CCAATCTXT
```

Sa1 clone Sequenced with Rht-1o primer

Figure 2C-(6)

```
  1  CACGGTGTCC GTGGCGAGGT GGGTGGCGAA GCTGTCGTCG GGGGCGGCGC
 51  CGGCGCCCAC GCCGCCCATC CCCATGGCCA TCTCGAGCTG CTCCAGCTTC
101  TGCGCCACGT CCGCCATGTC GGAGGCGCGC ACCTTGTACC CGAGCGCCGC
151  CAGCAGCXCG XCCACCTCCT CCCCCTCCCC CGCCGCCGCC GACACCATCA
201  TCTTGTCCTC GGACGAXCCC ATGCCGCCAC CGCCGCCGCC GCTCCCTCCG
251  GCGTCCTGGT ACTCCCGCTT CATGATCCGC GAGCTACCTC GCCTCTCTAT
301  CTATCTCTGG CCAATAATTG CGCA
```

5a1 clone Sequenced with Rht-12 primer

Figure 2 C-(1)

```
  1  GACCACCACC GGCATGTAGT AATGTAATCC CTTCTTCXTT CCCAGTTCTC
 51  CACCGCCTCC ATGATCACCC GTAAAACTCC TAAGCCCTAT TATTACTACT
101  ATTATGTXTA AATGTCTATT ATTGCTAXGT GTAATTCCTC CAACCGCTCA
151  TATCAAAATA AGCACGGGCC GGACTTTGTT AGCAGCTCCA ATGAGAATGA
201  AATGAATTTT GTACGCAAGG CACGTCCAAA ACTGGGCTGA GCTTTGTTCT
251  GTTCTGTTAT GTTCATGGTG CTCACTGCTC TGATGAACAT GATGGTGCCT
301  CCAATGGGTG GCTTTGCAAT TGTTGAACGT TTTGGCTTGG GGGACTTGGT
351  GXXTGGTGCA TGGGAATGAA XATTCCACAT CCXCXGGAAT TAAAATTAGC
401  CCATCCCG
```

5a1 clone Sequenced with Primer Rht-13

Figure 3a

```
   1  TTTCAXTTTC XTCCTTTTTT CTTCTTTTTC CAACCCCCGG CCCCCXGACC
  51  CTTGGATCCA AATCCCGAAC CCGCCCCCAG AACCXGGAAC CGAGGCCAAG
 101  CAAAAGXTTT GCGCCAATTA TTGGCCAGAG ATAGATAGAG AGGCGAGGTA
 151  GCTCGCGGAT CATGAAGCGG GAGTACCAGG ACGCCGGAGG GAGCGGCGGC
 201  GGCGGTGGCG GCATGGGTTC GTCCGAGGAC AAGATGATGG TGTCGGCGGC
 251  GGCGGGGGAG GGGGAGGAGG TGGACGAGCT GCTGGCGGCG CTCGGGTACA
 301  AGGTGCGCGC CTCCGACATG GCGGACGTGG CGCAGAAGCT GGAGCAGCTC
 351  GAGATGGCCA TGGGGATGGG CGGCGTGGGC GCTGGCGCCG CCCCTGACGA
 401  CAGGTTXGCC ACCCGCXGGC CGCGGACACX GTGCAXTACA ACCCCACXGA
 451  CXTGTCGTCT TGGGTCGAGA GCATGCTGTC GGAGCTAAAX GAGCCGCXGC
 501  CGCCCCTCCC GCCCGCCCCG CAGCTCAACG CCTCCACCGT CACGGGCAGC
 551  GGCGGXTACT TXGATCTCCC GCCCTCAGTC GACTCCTCCA GCAGCATCTA
 601  CGCGCTGCGG CCGATCCCCT CCCCGGCCGG CGCGACGGCG CCGGCCGACC
 651  TGTCCGCCGA CTCCGTGCGG GATCCCAAGC GGATGCGCAC TGGCGGGAGC
 701  AGCACCTCGT CGTCATCCTC CTCATAXTCG TCTCTCGGTG GGGGCGCCAG
 751  GAGCTCTGTG GTGGAGGCXG CCCCGCCGGT CGCGGCCGCG GCCAACGCGA
 801  CGCCCGCGCT GCCGGTCGTC GTGGTCGACA CGCAGGAGGC CGGGATTCGG
 851  CTGGTGCACG CGCTGCTGGC GTGCGCGGAG GCCGTGCAGC AGGAGAACCT
 901  CTCCGCCGCG GAGGCGCTGG TGAAGCAGAT ACCCTTGCTG GCCGCGTCCC
 951  AGGGCGGCGC GATGCGCAAG GTCGCCGCCT ACTTCGGCGA GGCCCTCGCC
1001  CGCCGCGTCT TCCGCTTCCG CCCGCAGCCG GACAGCTCCC TCCTCGACGC
1051  CGCCTTCGCC GACCTCCTCC ACGCGCACTT CTACGAGTCC TGCCCCTACC
1101  TCAAGTTCGC GCACTTCACC GCCAACCAGG CCATCCTGGA GGCGTTCGCC
1151  GGCTGCCGCC GCGTGCACGT CGTCGACTTC GGCATCAAGC AGGGGATGCA
1201  GTGGCCCGCA CTTCTCCAGG CCCTCGCCCT CCGTCCCGGC GGCCCTCCCT
1251  CGTTCCGCCT CACCGGCGTC GGCCCCCCGC AGCCGGACGA GACCGACGCC
1301  CTGCAGCAGG TGGGCTGGAA GCTCGCCCAG TTCGCGCACA CCATCCGCGT
1351  CGACTTCCAG TACCGCGGCC TCGTCGCCGC CACGCTCGCG GACCTGGAGC
1401  CGTTCATGCT GCAGCCGGAG GGCGAGGAGG ACCCGAACGA AGAXCCCGAX
1451  GTAATCGCCG TCAACTCAGT CTTCGAGATG CACCGGCTGC TCGCGCAGCC
```

```
1501  CGGCGCCCTG GAAAAGGTTC TTGGGCACCG TGCGCCCCCG TGCGGCCCAG
1551  AATTCXTCAC CGTGGTGGAA ACAGGAGGCA AATCACAACT CCGGCACATT
1601  CCTGGACCGC TTCACCGAGT CTCTGCACTA CTACTCCACC ATGTTCGATT
1651  CCCTCGAGGG CGGCAGCTCC GGCGGCGGCC CATCCGAAGT CTCATCGGGG
1701  GCTGCTGCTG CTCCTGCCGC CGCCGGCACG GACCAGGTCA TXTCCGAGGT
1751  GTACCTCGGC CGGCAGATCT GCAACGTGGT GGCCTGCGAG GGGGCGGAAC
1801  GCACAGAXCG CCACGAGACG CTGGGCCAGT GGCGGAACCG GCTGGGCAAC
1851  GCCGGGTTCG AGACCGTCCA CCTGGGCTCC AATGCCTACA AGCAGGCGAX
1901  CACGCTGCTG GCGCTCTTCG CCGGCGGCGA ACGGCTACAX GTGGAAGAAA
1951  AGGAAGGCTG CCTGACGCTG GGGTTGCACA CXCCCCCCTG ATTGCCACCT
2001  CGGCATGGCG CCTGGCCGGG CCGTGATCTC GCGAGTTTTG AACGCTGTAA
2051  GTACACATCG TGAGCATGGA GGACAACACA GCCCCGGCGG CCGCCCCGGC
2101  TCTCCGGCGA ACGCACGCAC GCACGCACTT GAAGAAGAAG AAGCTAAATG
2151  TCATGTCAGT GAGCGCTGAA TTGCAGCGAC CGGCTACGAT CGATCGGGCT
2201  ACGGGTGGTT CCGTCCGTCT GGCGTGAAGA GGTGGATGGA CGACGAACTC
2251  CGAGCCGACC ACCACCGGCA TGTAGTAATG TAATCCCTTC TTCGTTCCCA
2301  GTTCTCCACC GCCTCCATGA TCACCCGTAA AACTCCTAAG CCCTATTATT
2351  ACTACTATTA TGTTTAAATG TCTATTATTG CTATGTGTAA TTCCTCCAAC
2401  CGCTCATATC AAAATAAGCA CGGGCCGGAC TTTGTTAXCA GCTCCAATGA
2451  GAATGAAATG AATTTTGTAC GCAAGGCACG TCCAAAACTG GGCTGAGCTT
2501  TGTTCTGTTC TGTTATGTTC ATGGTGCTCA CTGCTCTGAT GAACATGATG
2551  GTGCCTCCAA TGGTGGCTTT GCAATTGTTG AAACGTTTGG CTTGGGGGAC
2601  TTGXGTGGGT GGGTGCATGG GGATGAATAT TCACATCXCC GGATTAAAAT
2651  TAAGCCATCC CGTTGGCCGT CCTTTGAATA XCTTGCCCXA AACGAAATTT
2701  CCCCCXATC
```

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Figure 3b

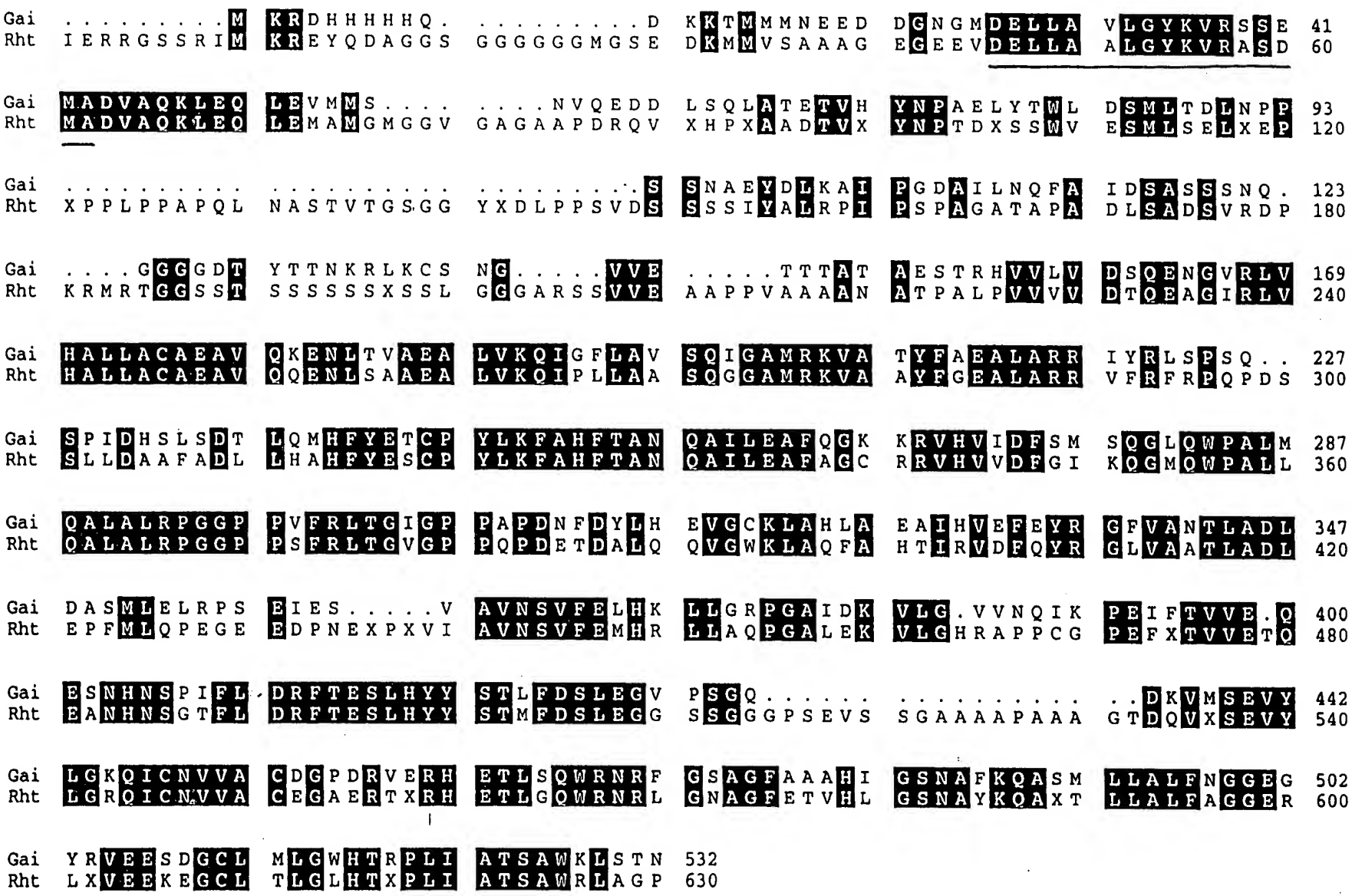

Figure 4a

```
  1  ACGCGTCCGG AAGCCGGCGG GAGCAGCGGC GGCGGGAGCA GCGCCGATAT
 51  GGGGTCGTGC AAGGACAAGG TGATGGCGGG GGCGGCGGGG GAGGAGGAGG
101  ACGTCTACGA GCTGCTGGCG GCGCTCGGGT ACAAGGTGCG GTCGTCCGAC
151  ATGGCCGACG TCGCGCAGAA XCTGGAGCAG CTGGAGATGG CCATGGGGAT
201  GGGCGGCGTG AGCGCCCCCG GCGCCGCGGA TGACGGGTTC GTGTCGCACC
251  TGGCCACGGA CACCGTGCAC TACAACCCCT CGGACCTCTC CTCCTGGGTT
301  CXGAGAGCAT GCTTTCGGAG TTAAAGGCGC CGTTGCCCCT TATCCCGCCA
351  GGCGCCGCCG GGCTGCCCGC CATGCTTTCC AACTTCGTCC ACTGTCACCG
401  GCGGCGGTGG TAGCGGCTTC TTTGAAXTCC CAGCCGCTGC CGAXTCGTCG
451  AGTAGCACXT ACGCCCTCAG GCCGATCTCC TTACCGGTGG TGGCGACGGC
501  TGACCCGTCG GCTGCTGACT CGGCGAGGGA CACCAAGCGG ATGCGCACTG
551  GCGGCGGCAG CACGTCGTCG TCCTCATCGT CGTCTTCCTC TCTGGGCGGT
601  GGGGCCTCGC GGGGCTCTGT GGTGGAGGCT GCTCCGCCGG CGACGCAAGG
651  GGCCGCGGCG GCGAATGCGC CCGCCGTGCC GGTTGTGGTG GTTGACACGC
701  AGGAGGCTGG XATCGGGCCT GGTGC
```

Figure 46

```
Wheat  IERRGSSRIM KREYQDAGGS GGGGGGMGSE DKMMVSAAAG EGEEVDELLA ALGYKVRASD  60
Rice   .........  TRPEAGGSSG GGSSADMGSC KDKVMAGAAG EEEDVDELLA ALGYKVRSSD  50
Gai    .........M KRDHHHHHQD .......... KKTMMMNEED DGNGMDELLA VLGYKVRSSE  41

Wheat  MADVAQKLEQ LEMAMGMGGV GAGAAPDRQV XHPXAADTVX YNPTDXSSWV ESMLSELXEP 120
Rice   MADVAQxLEQ LEMAMGMGGV SAPGAADDGF VSHLATDTVH YNPSDLSSWV ESMLSELKAP 110
Gai    MADVAQKLEQ LEVMMS.... ....NVQEDD LSQLATETVH YNPAELYTWL DSMLTDLNPP  93

Wheat  XPPLPP.APQ LNA......S TVTGSG..GY XDLPPSVDSS SSIYALRPIP SPAGATAPAD 171
Rice   LPLIPPGAAG LPAMLSPTSS TVTGGGGSGF FExPAAAxSS SSTYALRPIS LPVVATADPS 170
Gai    .......... .......... .......... ........SS NAEYDLKAIP GDAILN...Q 112

Wheat  LSADSVRDPK RMRTGGSSTS SSSSSXSSLG GGA.RSSVVE AAPPV..AAA ANATPALPVV 228
Rice   .AADSARDTK RMRTGGGSTS SSSSSSSSLG GGASRGSVVE AAPPATQGAA AANAPAVPVV 229
Gai    FAIDSA.... .......... .SSSNQGGGG DTYTTNKRLK CSNGVVETTT ATAESTRHVV 157

Wheat  VVDTQ..EAG IRLVHALLAC AEAVQQENLS AAEALVKQIP LLAASQGGAM RKVAAYFGEA 286
Rice   VVDTQEEEAG IRLVHALLAC XEAVQQENR. .......... .......... .......... 258
Gai    LVDSQ..ENG VRLVHALLAC AEAVQKENLT VAEALVKQIG FLAVSQIGAM RKVATYFAEA 215

Wheat  LARRVFRFRP QPDSSLLDAA FADLLHAHFY ESCPYLKFAH FTANQAILEA FAGCRRVHVV 346
Rice   .......... .......... .......... .......... .......... .......... 258
Gai    LARRIYRLSP SQ..SPIDHS LSDTLQMHFY ETCPYLKFAH FTANQAILEA FQGKKRVHVI 273

Wheat  DFGIKQGMQW PALLQALALR PGGPPSFRLT GVGPPQPDET DALQQVGWKL AQFAHTIRVD 406
Rice   .......... .......... .......... .......... .......... .......... 258
Gai    DFSMSQGLQM PALMQALALR PGGPPVFRLT GIGPPAPDNF DYLHEVGCKL AHLAEAIHVE 333

Wheat  FQYRGLVAAT LADLEPFMLQ PEGEEDPNEX PXVIAVNSVF EMHRLLAQPG ALEKVLGHRA 466
Rice   .......... .......... .......... .......... .......... .......... 258
Gai    FEYRGFVANT LADLDASMLE LRPSEIES.. ...VAVNSVF ELHKLLGRPG AIDKVLG.VV 387

Wheat  PPCGPEFXTV VETQEANHNS GTFLDRFTES LHYYSTMFDS LEGGSSGGGP SEVSSGAAAA 526
Rice   .......... .......... .......... .......... .......... .......... 258
Gai    NQIKPEIFTV VE.QESNHNS PIFLDRFTES LHYYSTLFDS LEGVPSGQ.. .......... 434

Wheat  PAAAGTDQVX SEVYLGRQIC NVVACEGAER TXRHETLGQW RNRLGNAGFE TVHLGSNAYK 586
Rice   .......... .......... .......... .......... .......... .......... 258
Gai    ......DKVM SEVYLGKQIC NVVACDGPDR VERHETLSQW RNRFGSAGFA AAHIGSNAFK 488
```

Figure 4b (cont

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```
Wheat  QAXTLLALFA  GGERLXVEEK  EGCLTLGLHT  XPLIATSAWR  LAGP630
Rice   ..........  ..........  ..........  ..........  ....258
Gai    QASMLLALFN  GGEGYRVEES  DGCLMLGWHT  RPLIATSAWK  LSTN532
```

Figure 3

Primers used in the RHT sequencing project.

Name	Sequence	Sense
RHA-1	CTG GTG AAG CAG ATA CCC TTG C	Forward
RHA-2	CTG GTT GGC GGT GAA GTG CG	Reverse
RHA-3	GCA AGG GTA TCT GCT TCA CCA GC	Reverse
RHA-5	CGC ACT TCA CCG CCA ACC AG	Forward
RHA-7	CCG TGC GCC CCC GTG CGG CCC AG	Forward
RHA-8	AGG CTG CCT GAC GCT GGG GTT GC	Forward
RHT-9	GAT CGG CCG CAG CGC GTA GAT GC	Reverse
RHT-10	GAT CCC GCA CGG AGT CGG CGG ACA G	Reverse
RHT-12	TCC GAC AGC ATG CTC TCG ACC CAA G	Reverse
RHT-13	TTC CGT CCG TCT GGC GTG AAG AGG	Forward
RHT-14	AAA TCC CGA ACC CGC CCC CAG AAC	Forward
RHT-15	GCG CCA ATT ATT GGC CAG AGA TAG	Forward
RHT-16	GGC ATG GGT TCG TCC GAG GAC AAG	Forward

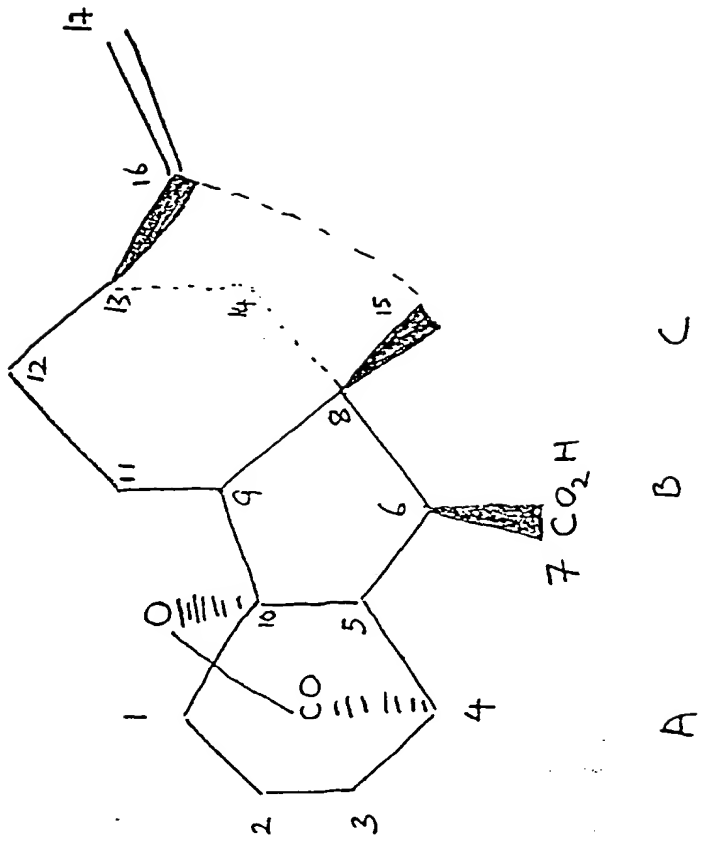

Figure 6

① 98102383

② 7 Aug 08

③ Melaburn. Ellis.